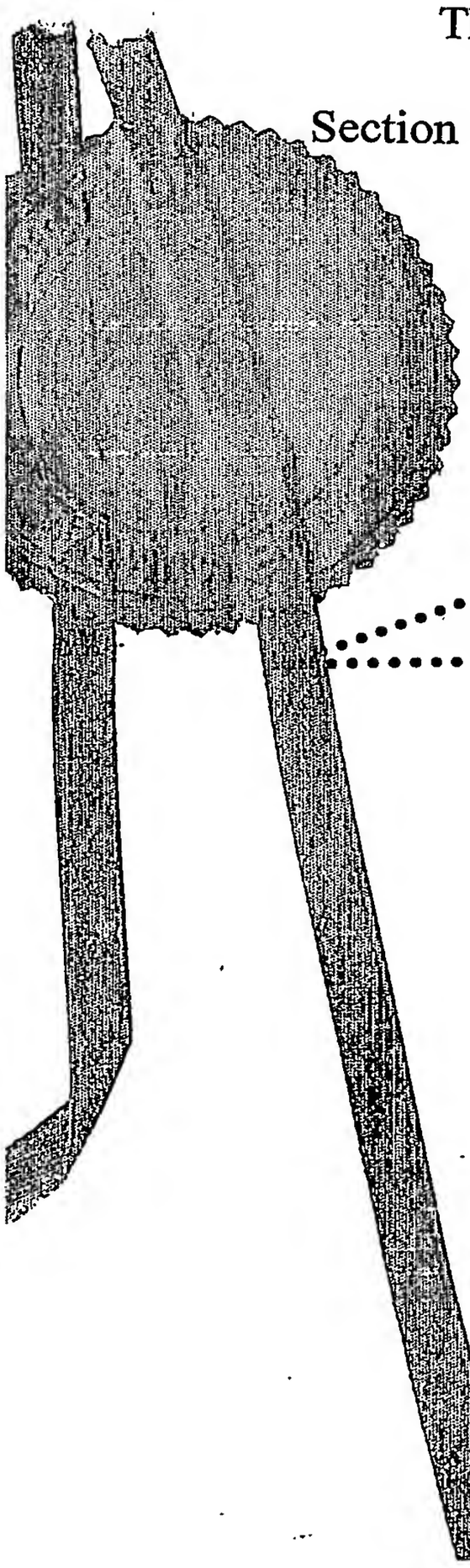
PCT / IN 03 / 00345

THE PATENTS ACT, 1970

IT IS HEREBY CERTIFIED THAT, the annex is a true copy of Application and provisional specification filed on 29.10.2002 in respect of Patent Application No. 937/MUM/2002 of Lupin Limited, a company incorporated under the Companies Act, 1956, of 159 CST Road, Kalina, Santacruz (East), Mumbai – 400 098, Maharashtra, India.

This certificate is issued under the powers vested in me under Section 147 (1) of the Patents Act, 1970.



Dated this 6th day of November 2003

M.A. Haafeez.

(M.A. HAAFEEZ)
ASST. CONTROLLER OF PATENTS & DESIGNS

FORM 1

THE PATENTS ACT, 1970

APPLICATION FOR GRANT OF PATENT
(See Sections 5(2), 7, 54 and 135 and Rule 33A)

(1) We, LUPIN LIMITED, a Company incorporated under the Companies Act, 1956, of 159 CST Road, Kalina, Santacruz (East), Mumbai - 400 098, Maharashtra, India

(2) hereby declare –

(a) That we are in possession of an invention titled

"AN IMPROVED METHOD FOR PREPARATION OF CEFTIOFUR"

(a) that the Complete/Provisional Specification relating to this invention is filed with this application;

(b) that there is no lawful ground of objection to the grant of a patent to us.

(3) Further declare that the inventors for the said invention are:

(1) **TYAGI, Om Dutt**; (2) **RICHHARIYA, Santosh Kumar**; (3) **PAWAR, Rajesh Kumar Ramchandra**; and (4) **CHAVAN, Yuvaraj Atmaram**; all Indian citizens of Lupin Limited (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune-411 042, Maharashtra, India

(4) We claim priority from the application filed in the following convention country, particulars of which are as follows:

NIL

(5) That we are the assignees of the true and first inventors.

ORIGINAL

(6) That our address for service in India is as follows:

937/MUM/2002
29/10/2002

SUBRAMANIAM, NATARAJ & ASSOCIATES
Attorneys-at-Law
E 556, Greater Kailash II,
New Delhi - 110 048, India.
Phone: 91 11 628 5603/6012/6025
Facsimile: 91 11 6286005
Email: sna@vsnl.com

Received Rs. 5500/-
Cash/M.O./P.O./Cq. 29/10/02
Vide Entry No. 1452 in the Register of Valuables, Bombay.

(7) Following declaration was given by the inventors:

We, (1) **TYAGI, Om Dutt**; (2) **RICHHARIYA, Santosh Kumar**; (3) **PAWAR, Rajesh Kumar Ramchandra**; and (4) **CHAVAN, Yuvaraj Atmaram** all Indian citizens of Lupin Limited (Research Park), 46A/47A, Nande Village, Taluka Mulshi, Pune-411

042, Maharashtra, India, the true and first inventor for this application declare that the applicants here are our assignees.

TYAGI, Om Dutt

RICHHARIYA, Santosh Kumar

PAWAR, Rajesh Kumar Ramchandra

CHAVAN, Yuvaraj Atmaram

(8) That to the best of our knowledge, information and belief the facts and matters stated herein are correct and there is no lawful ground of objection to the grant of patent to me/us on this application.

(9) Following are the attachments with this application:

(a) Provisional specification in triplicate
(b) Application forms 1 in triplicate
(c) Statement and Undertaking on FORM 3 in duplicate
(d) Drawings in triplicate
(e) Abstract

Fee Rs. ..... in Cash/Cheque/Bank Draft Bearing No..... dated.....on .....Bank.

We request that a patent be granted to us for the said invention.

Dated this 26th day of October 2002

LUPIN LIMITED

The Controller of Patents
The Patent Office,
At Mumbai

Form 2

THE PATENTS ACT, 1970

PROVISIONAL SPECIFICATION
(Section 10)

"AN IMPROVED METHOD FOR THE PREPARATION OF CEFTIOFUR"

ORIGINAL

LUPIN LIMITED, a company organised and existing under the Companies Act, 1956, of 159 CST Road, Kalina, Santacruz (East), Mumbai-400 098, Maharashtra

The following specification describes the nature of the invention:

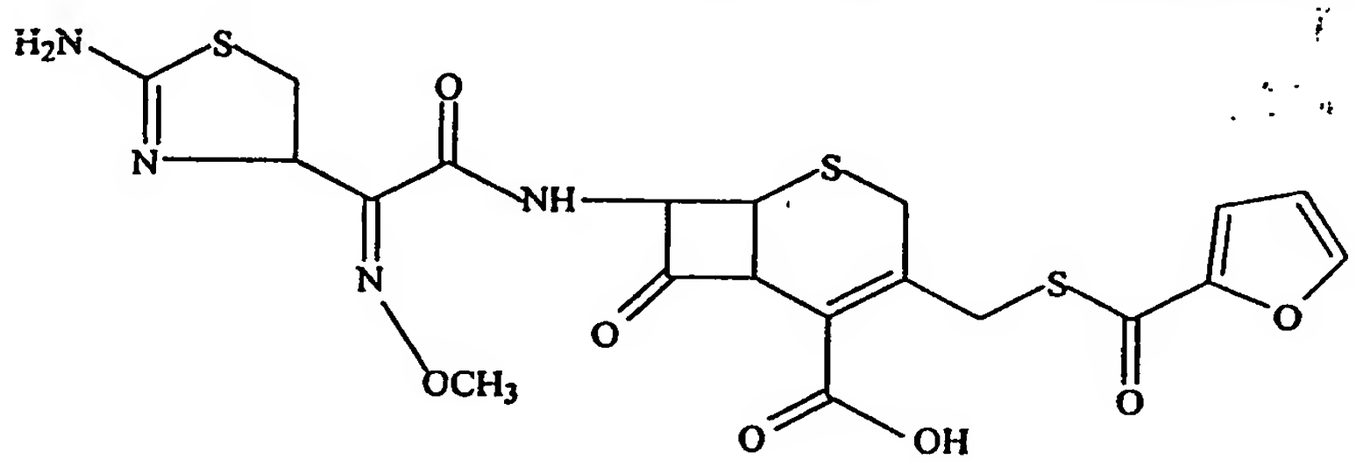
An Improved process for preparation of Ceftiofur

Field of Invention

The present invention relates to an improved process for preparation of ceftiofur of formula (I).

Background of the invention

Ceftiofur is a broad spectrum third generation antibiotic, which is primarily used for veterinary use. It is known chemically as (6R, 7R)-7-[[(2Z)-(2-amino-4-thiazolyl) (methoxyimino) acetyl] amino]-3-[[(2-furanylcarbonyl) thio] methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid and is represented by the formula (I).



(I)

US Patent No. 4,464,367 (Labeeuw et.al) describes the preparation of ceftiofur (I) comprising,

a) reaction of 7-ACA (II) with thiofuroic acid (III) to give the 3-thiomethyl derivative (IV) which on reaction with a suitably activated [(2Z)-(2-tritylamino-4-thiazolyl)methoxyimino] acetic acid of formula (V), in the presence of dicyclohexylcarbodiimide and subsequent deprotection gives ceftiofur (I).

b) reaction of a compound of formula (VI), i.e. cefotaxime acid with thiofuroic acid (III) to give ceftiofur (I).

Suitable activation of the carboxylic acid moiety of [2-(2-tritylaminothiazol-4-yl)-2-syn-methoxyimino acetic acid is carried out with 1-hydroxy benzothiazole (HOBT).

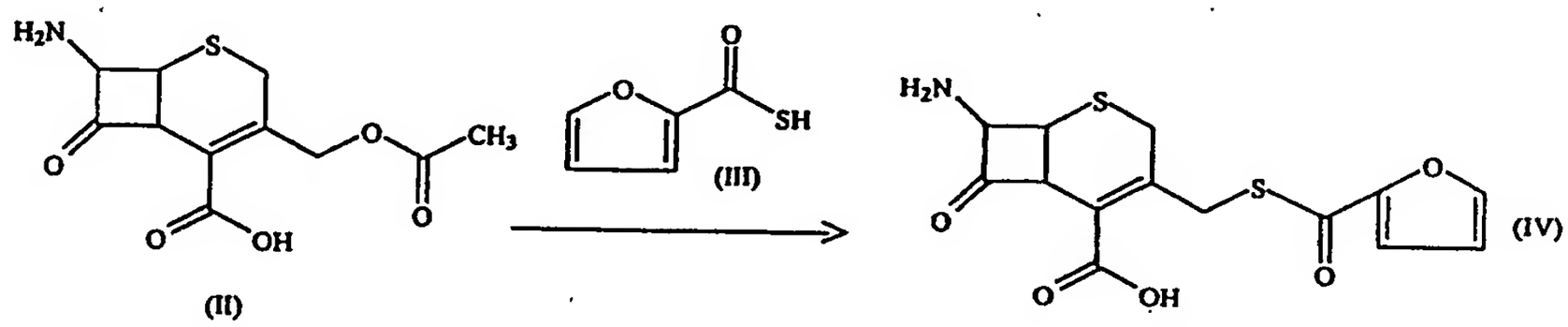
However, apart from the schematic reaction sequence representation, no enabling

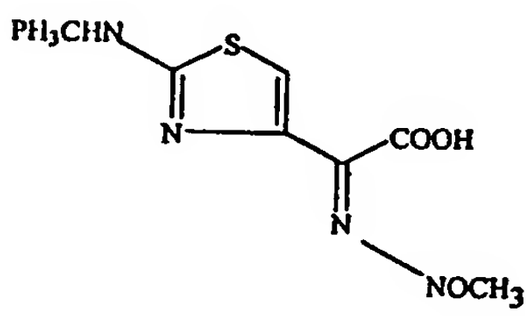
conditions for the conversion of cefotaxime acid (VI) to ceftiofur (I) has been described in the aforesaid patent.

The above mentioned methods for preparation of ceftiofur (I) is summarized in scheme-I.

Scheme-1

a)





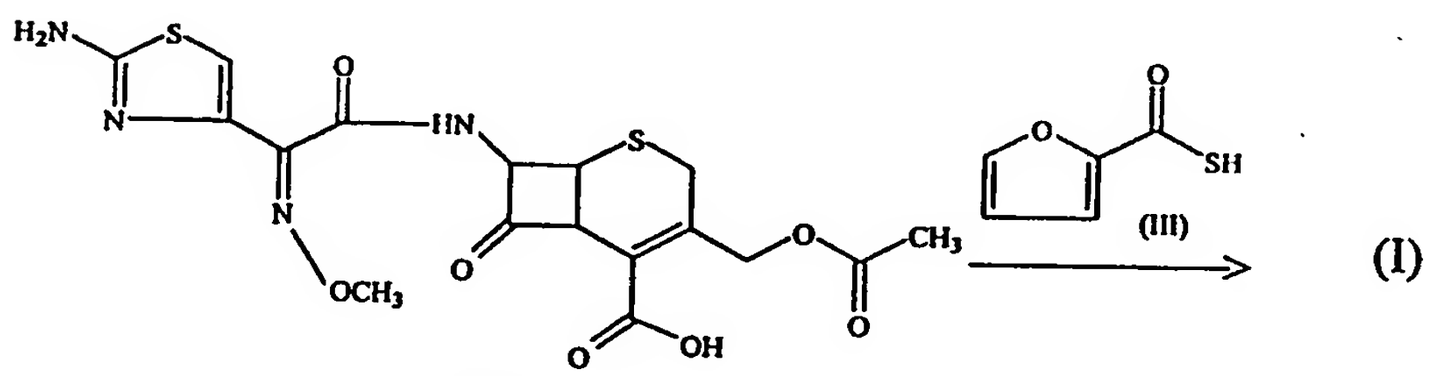
(I)

a)Acid activation

b)Condensation

c)Deprotection

b)



(I)

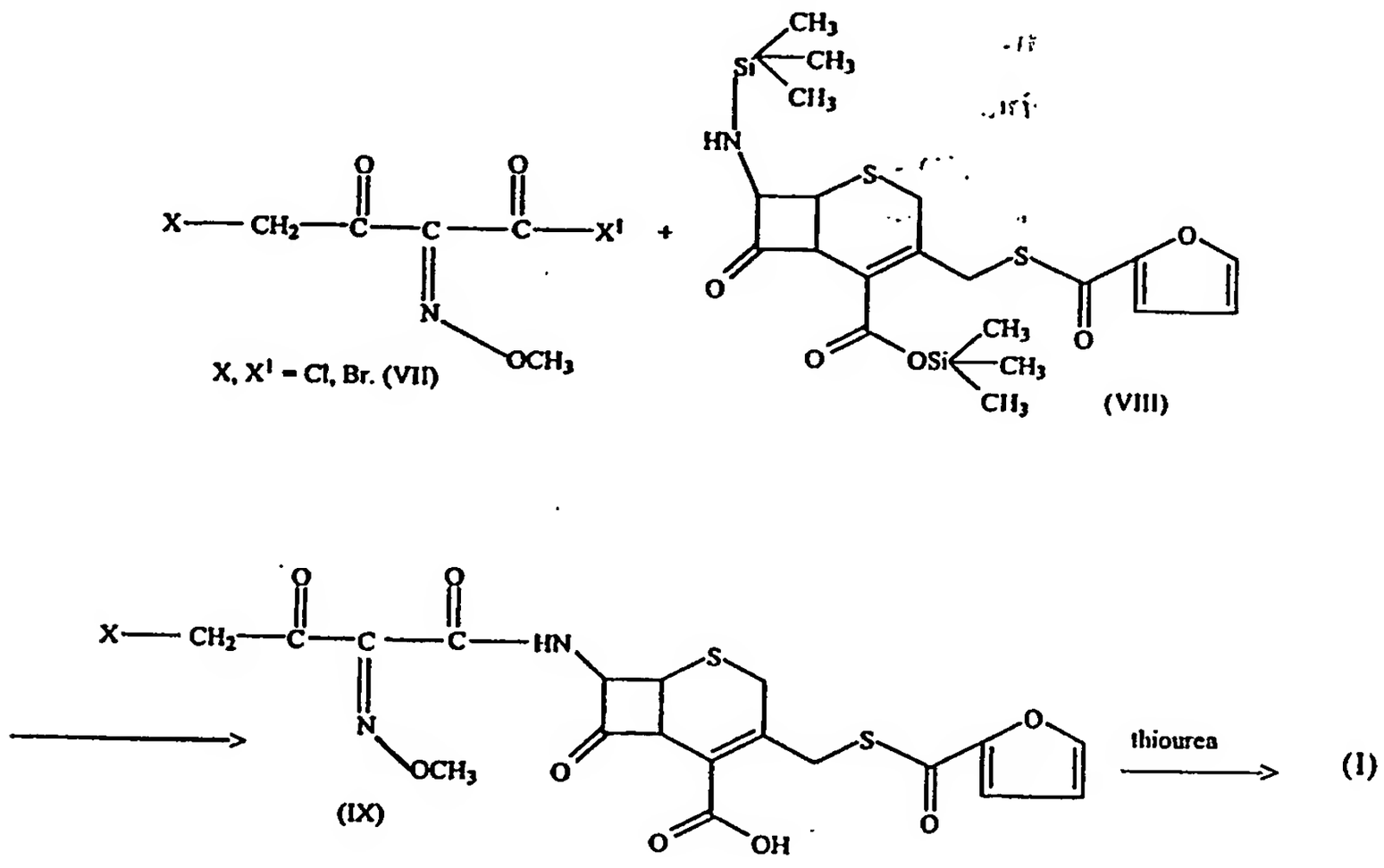
(VI)

US patent No.6, 458, 949 B1 (Handa. et. al) describes a method for preparation of ceftiofur (I), which is summarized in scheme (II). The method comprises of

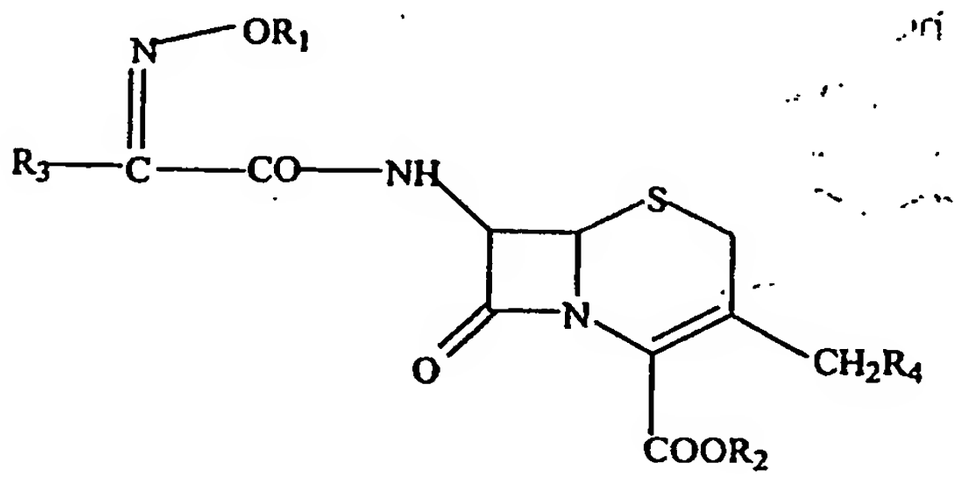
a) reacting 4-halo-3-oxo-2-methoxyimino butyric acid, activated as the acid halide (VII), with silylated 7-amino-3-thiomethyl furoyl-3-cephem-4-carboxylic acid (VIII), to give the corresponding 7-carboxamido derivative (IX) which subsequently on treatment with thiourea gives ceftiofur of formula (I).

The process is not only lengthy, but the yields obtained are also low, rendering the method commercially not very attractive.

Scheme-II



US Patent No. 4,767,852 (Ascher, et.al) teaches a method for the acylation of 7-amino-3-cephem-4-carboxylic derivatives with the benzothiazole thioester of [2-(2-aminothiazol-4-yl)]-2-syn-methoxyimino acetic acid (MAEM), to give the corresponding 7-acylamido cephalosporin of formula (X).



(X)

wherein, R_1 is hydrogen, alkyl, phenyl alkyl, carbalkoxyalkyl, acyl or carboxyalkyl.

R_2 is H, pivaloyloxymethyl or a carboxy protecting group,

R_3 is a five- membered oxygen or sulphur containing heterocyclic ring, which may be substituted by amino or azido, and

R_4 is H, acetoxy, carbamoyloxy, or –S-Y, in which Y is a heterocyclic ring, which may be substituted.

when R_4 is acetoxy and R_3 is [2-(2-aminothiazol-4-yl)-2- syn- methoxyimino acetic acid, the compound relates to cefotaxime,

when R_4 is S-Y, and Y is 2,5-dihydro-6-hydroxy-2-methy-5-oxo-triazin-3-yl, and R_3 is [2-(2-aminothiazol-4-yl)-2-syn-methoxyimino acetic acid, the compound relates to ceftriaxone.

when R_4 is S-Y, and Y is –1,2,3- thiadiazol-5-yl and R_3 is [2-(2-aminothiazol-4-yl)-2-syn-methoxyimino acetic acid, the compound relates to cefuzonam.

The aforesaid patent essentially, encompasses only those 3-thiomethyl compounds in which the sulphur atom is directly attached to a heterocylic ring, but does not include those compounds in which a carbonyl group is interposed between S and Y, where Y is a heterocyclic ring.

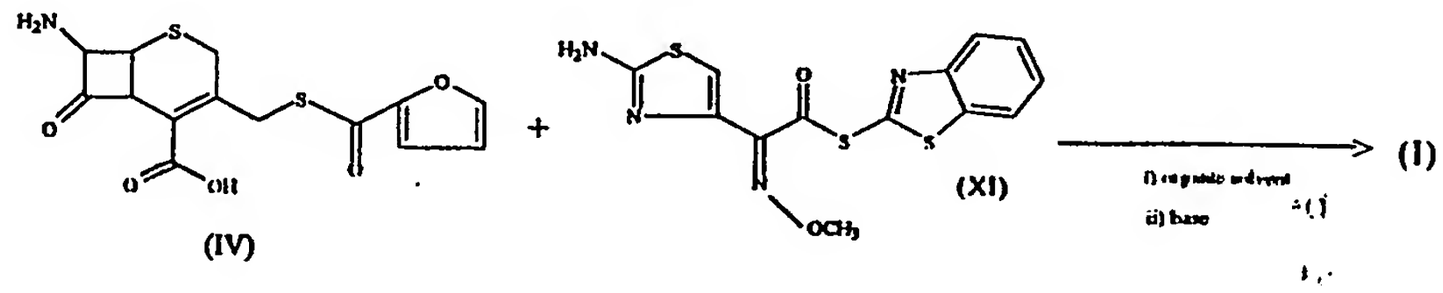
Summary of the invention:

The object of the invention is to provide an improved industrial process for preparation of ceftiofur (I) in good yield and purity.

Accordingly, the present invention provides a process for preparation of ceftiofur of formula (I) comprising reaction of 7-amino-3-thiomethyl furoyl-3-cephem-4-carboxylic acid (IV) with [2-(2-aminothiazol-4-yl)-2-syn-methoxyimino] acetic acid activated as the mercaptobenzothiazole thioester (XI), (commonly referred to as MAEM) to give ceftiofur with high yield and purity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention describes a process for preparation of ceftiofur of formula (I) comprising, reaction of, 7-amino-3-thiomethyl furoyl-3-cephem-4-carboxylic acid of formula (IV) and [2-(2-aminothiazol-4-yl)]-2- syn- methoxyimino acetic acid –2-benzothiazolyl thioester (MAEM) (XI).



(IV) (XI) (I)

The process comprises reaction of 7-amino-3-thiomethyl furoyl-3-cephem-4-carboxylic acid (IV) with 2-mercapto benzothiazole thioester of [2-(2-aminothiazol-4-yl)]-2-synmethoxyimino acetic acid (XI), (referred to as MAEM) in the presence of an organic solvent and an organic base, and optionally in the presence of water. The reaction is monitored by HPLC, and worked up by washing with a water–immiscible solvent as herein described, precipitating the product in a biphasic system by adjusting the pH, isolating and drying the product having the formula (I).

Preferably the reaction between MAEM (XI) and compound (IV) is carried out at a temperature ranging from –5 to –15°C, for about 3 to 6 hours.
The organic solvent used is selected from chlorinated hydrocarbons, tetrahydrofuran, or a mixture thereof or only water. Preferably the reaction is carried out in dichloromethane or water. The reaction is carried out in the presence of organic bases like triethyl amine, dicyclohexyl amine etc or a mixture thereof.
The water immiscible solvents used are chlorinated hydrocarbons.
Ceftiofur having the formula (I) prepared according to the present invention is a syn-isomer of this compound.

The invention can be further illustrated by the following examples, which however should not be construed to be limiting the scope of the invention.

Example 1: Preparation of ceftiofur (I).

7-amino-3-(2-furanylcarbonylthiomethyl)-3-cephem-4-carboxylic acid (IV) (50gms;0.1470moles) was added to dichloromethane(750ml). Dichloromethane (250ml) was distilled, and the reaction mixture cooled to 0°C. Triethyl amine (29.76gms; 0.294 moles) was added at 0°C in 60 minutes. MAEM (61.76gms; 0.177moles) followed by methanol (50 ml), was added at 5-7°C. The reaction was monitored by HPLC and reaction mixture stirred till the starting material was ≤ 1.0% on HPLC. The reaction mixture was worked up by adding water (400ml) and stirred for 15 minutes at 10-15°C. The layers were separated and the aqueous layer was washed with dichloromethane (250ml). The aqueous layer was treated with carbon and filtered. Ethyl acetate (150ml) was added to the filtrate and pH adjusted to 3.0 by addition of 15% orthophosphoric acid in 45 minutes at 25-30°C. The product was filtered and washed with water (200ml) followed by ethyl acetate (200ml). The product was dried at 30°C under reduced pressure to give 53.5 gms (69.57%) of the title compound.

Example 2

Preparation of ceftiofur (I).

Compound (IV) (25gms;0.0735 moles) was added to a mixture of tetrahydrofuran (175ml) and water (250ml) at ambient temperature and cooled to 0°C. Triethyl amine (9.58gms;0.095 moles) was added slowly to the mixture in 60 minutes. MAEM (29.59gms;0.0845 moles) was added and the reaction mixture was stirred at 0°C, for 8.0hours. The reaction mixture was monitored by HPLC and stirred till all the starting material was consumed. The reaction mixture was worked up by extracting with dichloromethane (750ml). The aqueous layer after carbon treatment was diluted with isopropyl alcohol (75ml) and methyl isobutyl ketone (37.5ml). The pH of the aqueous mixture was adjusted to 3.0 by addition of 15% orthophosphoric acid. The product was filtered and washed with water (250ml). The product was dried at 30°C, under reduced pressure to give 25.6 gms (66.5%) of the title compound.

Dated this the 26th day of October 2002.

H. SUBRAMANIAM
Attorney for the applicants